



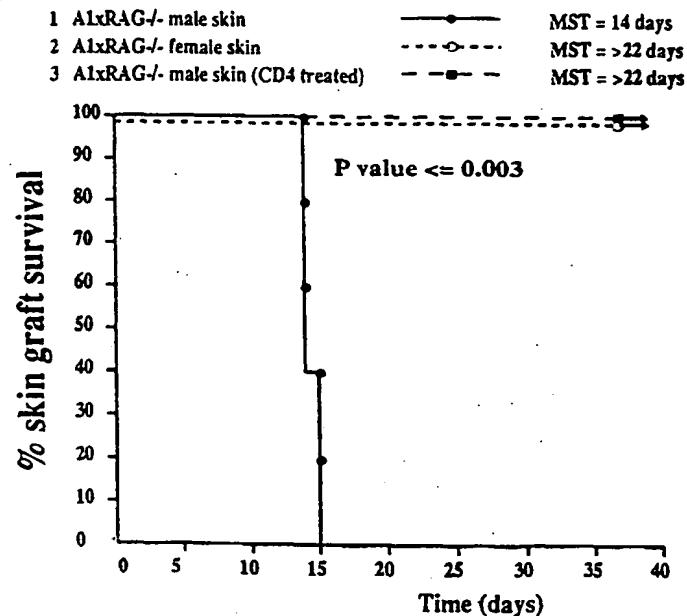
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: C12N 15/00, A01K 67/027, C07K 14/705	A1	(11) International Publication Number: WO 99/16867 (43) International Publication Date: 8 April 1999 (08.04.99)
<p>(21) International Application Number: PCT/GB98/02965</p> <p>(22) International Filing Date: 30 September 1998 (30.09.98)</p> <p>(30) Priority Data: 9720888.8 1 October 1997 (01.10.97) GB</p> <p>(71) Applicant (for all designated States except US): ISIS INNOVATION LIMITED [GB/GB]; 2 South Parks Road, Oxford, Oxfordshire OX1 3UB (GB).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): WALDMANN, Herman [GB/GB]; Sir William Dunn School of Pathology, South Parks Road, Oxford, Oxfordshire OX1 3RE (GB). COBBOLD, Stephen, Paul [GB/GB]; Sir William Dunn School of Pathology, South Parks Road, Oxford, Oxfordshire OX1 3RE (GB). ZELENIKA, Diana [FR/GB]; Sir William Dunn School of Pathology, South Parks Road, Oxford, Oxfordshire OX1 3RE (GB).</p> <p>(74) Agents: PRIVETT, Kathryn, L. et al.; Stevens, Hewlett & Perkins, 1 Serjeants' Inn, Fleet Street, London EC4Y 1LL (GB).</p>		<p>(81) Designated States: JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> </p>

(54) Title: TRANSGENIC MODEL COMPRISING TCR ALPHA AND BETA CHAINS

(57) Abstract

The invention provides a genetically modified non-human mammal having a population of CD4 positive T cells specific for one or a limited number of selected antigens, including at least one transplantation antigen capable of rejecting a tissue transplant containing the transplantation antigen and if applicable the other selected antigens. A genetically modified animal according to the invention has T cell receptor genes which encode a T cell receptor specific for the transplantation antigen. The genetically modified mammal is useful in studying immunological tolerance, in particular in the mechanisms of tolerance to and the rejection of tissue grafts, and in pregnancy. The animals are also useful for testing agents for biological activity in promoting or reducing immunological tolerance.



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TRANSGENIC MODEL COMPRISING TCR ALPHA AND BETA CHAINS

This invention relates to a genetically modified non-human
5 mammal having limited T cell receptors, capable of mounting an immune response to only one or to only a limited number of antigens, and of being tolerised to the antigen or antigens. The invention also relates to methods of testing biologically active compounds using such a genetically modified mammal.

10 Immunological tolerance is a state of unresponsiveness of the immune system which is specific for a particular antigen. An important aspect of tolerance is self-tolerance, which is an unreactiveness to self that allows the body to distinguish between self antigens presented to it on normal tissues and potentially dangerous situations such as infection. It is
15 a generally held view that the immune system is unreactive to self antigens, but maintains a broad repertoire of receptors sufficient to recognise any non-self antigen presented to it (central tolerance). It is also generally accepted that there must be some fail-safe mechanisms that operate to minimise the effects of any mistakes that may lead to
20 autoreactivity (peripheral tolerance). The possible mechanisms for tolerance, in particular for peripheral tolerance, are reviewed in Cobbold et al. 1996 Immunol. Rev. 149:5-33.

Tolerance is clearly a matter of practical importance in treatments which require the introduction of foreign antigens into the body,
25 such as tissue transplantation and vaccination, and in diseases which are characterised by immunosuppression such as AIDS or by a breakdown in self-tolerance resulting in autoimmunity. However, the techniques for studying tolerance and for investigating the effects of pharmaceutical compounds on tolerance have until now been limited.

Until relatively recently, neonatal tolerance induction was the primary technique for experimental study of tolerance. Specific tolerance to grafted skin was induced in mice by neonatal injection of spleen cells from a different strain. The resulting mice showed tolerance to skin grafts 5 from the donor strain. Moving on from the neonatal induction models, transgenic methods for introducing foreign transgenes into mice have allowed the study of specific antigens in a defined genetic background which are treated as self by the immune system.

Techniques for inducing tolerance in the mature adult 10 immune system have also been developed. Monoclonal antibodies against the murine CD4 and CD8 antigens are a powerful means to produce tolerance to a variety of different antigens in the adult mouse. Depletion of the CD4 or CD8 T cells is not necessary; a blockading with either (Fab¹)₂ fragments or a non-depleting isotype of anti-CD4 or anti-CD8 antibody is 15 especially effective. Combinations of CD4 as well as CD8 antibodies can be used for the extended periods found necessary for inducing tolerance in some of the more difficult skin graft systems (Cobbold et al, 1990 Eur. J. Immunol. 20:2747-2755).

The discovery that tolerance could be induced to transplanted 20 organs under the cover of non-depleting CD4 and CD8 antibodies given together was a major breakthrough (e.g. Cobbold et al. 1990). Because skin grafts can be used as both the tolerogen and later test challenge, the system gives a consistent read-out for rejection/tolerance *in vivo* that does not depend on making assumptions about the effector cells or the 25 antigens, as is necessary for *in vitro* measurements. The main advantage of this approach over that of neonatal tolerance induction, or the introduction of foreign transgenes, is that it makes it possible to focus on events that take place without the thymus (by removing it) and without other previous exposure to the antigen during the development of the 30 immune system. This allows more precise control of the timing of

tolerance induction and antigen challenge. There is also a wider choice of how the antigen is presented for tolerance by using different organs as grafts.

However, in all of the techniques described, there is a limited
5 amount of specific information that can be gained relating to the mechanisms for rejection and tolerance, because of the complexity of the immune system and the immune response.

It is therefore an object of the invention to provide a simplified system for observing an immune response and/or tolerance to a particular
10 antigen or to a limited number of antigens.

It is another object of the invention to provide a system for testing the effects of treatments on an immune response and/or tolerance to a particular antigen or to a limited number of antigens.

Surprisingly, it has been found that mice having only CD4
15 positive T cells, all of which have a single T cell receptor specific for the same transplantation antigen, can reject skin grafts. Previously, it had been thought that the role of CD4 positive T cell in graft rejection was mainly to provide T cell help to either CD8 positive T cells to become cytotoxic, or to B cells to make antibody. Thus, CD8 positive T cell
20 receptor transgenic mice have been made (Hammerling et al. 1993 *Immunol. Rev.* 133:93-104). Furthermore, it was not expected that such a CD4 T cell receptor "monoclonal" mouse once it had been found to reject grafts, could be tolerised to the transplantation antigen. Previously, it was often assumed that tolerance works either by eliminating the specific T
25 cells (clonal deletion) or by inducing a separate population of suppressor cells. In fact, tolerance can be effectively induced by treatment with non-depleting anti-CD4 antibody.

The invention provides in one aspect a genetically modified non-human mammal having a population of CD4 positive T cells specific
30 for one or a limited number of selected antigens, including at least one

transplantation antigen, the mammal being capable of an immune response against the transplantation antigen and of being tolerised to the transplantation antigen.

Preferably, the genetically modified animal according to the invention is capable of rejecting a tissue transplant containing the transplantation antigen and if applicable the other selected antigens.

A genetically modified animal according to the invention has T cell receptor genes which encode a T cell receptor specific for the transplantation antigen. The T cell receptor genes will generally be stably integrated into its genome. Most T cell receptors are made up of α and β chains which are encoded by separate genes. The T cell receptor genes employed in the invention may be derived for example from cloned α and β chain genes for T cell receptors specific for the transplantation antigen, or from α and β chain genes prepared synthetically using sequence information from cloned genes. In another alternative, the genes are derived from α and β chain genes for T cell receptors which are not necessarily specific for the transplantation antigen. In that case, well known methods such as site-directed mutagenesis may be used to alter the gene sequences to achieve transplantation antigen specificity.

Preferably, the genetically modified animal according to the invention lacks a normal population of CD8 positive T cells, or B cells, or both. More preferably, the animal has no functional CD8 positive T cells or B cells. An absence of lymphocytes other than CD4 positive T cells provides a far simpler system for studying the activity of the immune system in relation to a selected transplantation antigen.

An absence of CD8 positive T cells and B cells may be as a result of the animal having a deficiency in lymphocyte receptor recombination such that no CD8 T cell receptors or B cell receptors (antibody) are expressed. For example, mice which are deficient in RAG-1 and/or RAG-2 (recombinase activating gene no. 1 and/or no. 2) activity

have an inability to initiate VDJ recombination in the lymphocyte receptor gene and therefore cannot generate mature lymphocytes. RAG mutations can therefore be used in the invention to eliminate functional lymphocytes other than the specific CD4 lymphocytes directed to the transplantation antigen (and specific CD4 lymphocytes directed to any other selected antigens). RAG-1^{-/-} mice are described in Alt et al. 1992 Ann. N. Y. Acad. Sci. 651:277-94 and Mombaerts et al. 1992 Cell 68(5):869-77.

The population of CD4 positive T cells which are specific for the transplantation antigen (or if appropriate, other selected antigens), will generally form the majority of the entire CD4 positive T cell population of the animal, for example 50-60% or more of the CD4 positive T cells. In the case of a recombination deficiency such as a RAG-deficiency, all functional CD4 positive T cells will be specific for the transplantation antigen (or other selected antigens).

The transplantation antigen to which the CD4 positive T cells are directed may be any transplantation antigen which is not expressed in the animal itself, or not expressed in the animal in a form recognisable by the CD4 positive T cells. A transplantation antigen is defined as a tissue antigen which can be introduced into the body in a tissue transplant in such a manner that it is recognised by the immune system. A tissue antigen is an antigen found on body tissues and organs. Tissue transplants for the purposes of the invention include foetuses in the maternal environment.

In a particular species there are characteristic transplantation antigens, each of which may be present or absent in any given individual, or any given group of individuals such as a strain, of the species. A transplantation antigen may occur in a variety of different forms such that it is recognisable as being foreign between different individuals or between different groups of individuals in the species. Transplantation antigens include major transplantation antigens such as the MHC (major histocompatibility complex) antigens, or minor transplantation antigens

such as for example the murine male transplantation antigen H-Y, the murine β_2 microglobulin antigen H-1, or mitochondrial antigens.

For certain transplantation antigens, in particular some of the minor transplantation antigens, CD4 positive T cells specific for a single 5 antigen may not be sufficient for rejection of a tissue graft. In the case of these minor transplantation antigens, further CD4 positive T cells having specificity for one or a few further antigens are provided, for example by stably integrating further specific T cell receptor (TCR) genes into the animal. The murine male transplantation antigen H-Y is an example of a 10 minor transplantation antigen which, conveniently, is sufficient on its own for graft rejection in an animal which has only H-Y antigen specific TCRs.

In another aspect, the invention provides a method of screening for biologically active compounds, which method comprises administering the compounds to a genetically modified non-human 15 mammal as described herein and observing the effect of the compounds on the ability of the mammal to reject or maintain a transplant containing the transplantation antigen.

In the case of a simple male/female transplantation antigen such as the male H-Y antigen, the method according to the invention can 20 employ a female recipient mouse and graft tissue from a congenic male donor mouse. Where a male or female transplantation antigen is not used, the recipient and donor animals are preferably from congenic strains with the donor strain being transgenic for the transplantation antigen. This means that the system is kept as simple as possible with the only antigenic 25 difference between the donor and recipient being the transplantation antigen (and other selected antigens if applicable).

The invention enables any specific changes in the T cell population, such as activation markers or cytokine production, to be easily monitored by conventional immunological techniques during 30 transplantation or during tolerance induction or maintenance, and also

during and after pregnancy. A so-called "Toleramouse" is thus an ideal tool for investigating the mechanisms of immunosuppression and tolerance induction. Previously, such experiments had to be performed either in normal inbred or congenic mice in which the frequency of T cells against any single antigen is too low for direct observation using simple immunological techniques such as immunofluorescent staining or cytokine staining assays.

The study of tolerance mechanisms in pregnancy may be performed on pregnant animals, in particular pregnant animals as described herein near to and after the end of pregnancy term. Particularly useful are pregnant females carrying one or more male foetuses displaying a male transplantation antigen, where the pregnant female has TCRs directed against the male transplantation antigen. Pregnant animals carrying male and female foetuses can be compared. The studies performed usefully include looking at the T cell population and T cell markers, including cytokine profiles.

It is envisaged that the invention will be particularly useful for testing potential or existing therapeutic agents:

- i) for wanted or unwanted tolerogenic or immunosuppressive (side) effects;
- ii) for interference with the tolerance process, either once it has been established or during the induction of tolerance by appropriate therapeutic agents such as CD4 monoclonal antibody.

More specifically, the invention may be used to screen for agents which:

- i) have tolerising effects in a similar manner to CD4 monoclonal antibodies;
- ii) do not interact with tolerance-inducing drugs;
- iii) enhance the immune response in diseases which feature immunosuppression such as AIDS and cancer;

iv) interact in desired ways with other drugs or with vaccines.

The invention is now further described in the following examples, which are not intended to limit the scope of the invention in any way.

5

EXAMPLES

Example 1 - Construction of Transgenic Mice

Methods

Mice:

10 CBA/Ca (Harlan/Olac, UK) mice were bred under specific pathogen free conditions and all experimental mice were maintained in the animal facility of the Sir William Dunn School of Pathology, Oxford, in a filtered cage system (Maximiser, Thorens Caging Inc., Hazelton PA, USA). RAG-1^{-/-} mice that had been bred onto an H-2^k background were obtained
15 from Dr. B. Stockinger (NIMR, London, UK).

Generation of A1(M) transgenic mice:

To generate transgenic mice we used the TCR α and β chain from the A1 CD4+ T cell clone isolated from CBA/Ca mice (Tonomari 1985. *Cell Immunol.* 96: 147-62). The A1 clone recognizes the minor histocompatibility antigen H-Y, present in male but absent in female mice, in the context of H2-E^k. The TCR expression was identified using primers specific for the Vα and Vβ gene families (Casanova et al. 1991. *J. Exp. Med.* 174(6): 1371-83), cloned and sequenced to check for productive rearrangement. The α chain was found to be encoded by LVα10-Jα30-Cα (EMBL accession no. AJ000157) and the β chain is encoded by LVβ5.1-Vβ8.2-Dβ2-Jβ2.3-Cβ2 (EMBL accession no. AJ000158) (Figure 1). EcoRI-EcoRI fragments containing the productively rearranged α or β chains were generated by RT-PCR. The oligonucleotides used for the
30 amplification of the α chain were :

GCGAATCACAAAGCACCATGAAGAGGGCTG [SEQ ID NO: 1] and
GCGAATTCCAGAC CTCAACTGGACCACAG [SEQ ID NO: 2]. The
oligonucleotides used for the amplification of the β chain were:
GCGAATTCAAGAGGAAGCATGTCTAACACT [SEQ ID NO: 3] and
5 GCGAATTCAAGGATGCATAAAAGT TTGTCTCAGG [SEQ ID NO: 4]. The
full length cDNAs were cloned into the human CD2 minigene (VA) in
pBluescript. Sall-XbaI fragments were used for microinjections into
CBA/Ca oocytes. Transgenic A1(M) founders were maintained on the
CBA/Ca background and bred to homozygosity. Southern blot analysis
10 indicated that the A1(M) line carries a single copy per haplotype of each of
the transgenic V α and V β chains.

Skin grafting

Pieces of tail skin approx. 0.5cm² were grafted onto the lateral
15 thoracic wall of anaesthetised recipient mice as previously described
(Cobbold and Waldmann 1986. *Transplantation* 41: 634-639; and Qin et al.
1990. *Eur. J. Immunol.* 20: 2737-2745). Where two grafts were given
simultaneously these were placed side by side in the same prepared graft
bed. Plaster casts were removed on day 7 and the grafts observed daily,
20 with rejection being defined as the day when no viable graft tissue could be
seen. Statistical significance was determined using the LogRank method
(Peto et al. 1977. *Br. J. Cancer* 35:1-39). All procedures were carried out
in accordance with the UK Home Office Animals (Scientific Procedures)
Act of 1986.

25

Immunofluorescent analysis and antibodies

Thymus, spleen or lymph nodes were removed, and
erythrocytes lysed by isotonic shock. Cells were labelled in phosphate
buffered saline (PBS) containing 0.1% (w/v) Na₃N₃, 1% (w/v) bovine serum
30 albumin (BSA), and 5% (v/v) heat inactivated normal rabbit serum (HINRS):

to block Fc receptors) at 4°C. Antibodies used were: CD3ε (KT3-FITC), Vβ8 (KJ16-FITC), Vβ8.2 (F23.2-FITC), CD4-PE (Sigma P2942), CD8α-QR (Sigma R3762), B220-QR (Sigma R4262), CD25 (PC61-biotin), CD44-QR (Sigma R5638), SA-APC (Pharmingen 13049A), and FITC goat anti-mouse IgG (Sigma F0257). After labelling and washing, cells were fixed in 1% formalin and stored in the dark at 4°C. Four colour analysis was performed using a FACSort (Becton Dickinson, Oxford, UK) with dual laser (488nm and 633nm) excitation together with data acquisition and cross-beam colour compensation using CellQuest 3.1 software. At least 50,000 events were stored in list mode for further analysis and gating on forward and side scatters.

Intracytoplasmic cytokine staining was performed using spleen cells given a 2 hr stimulation in vitro with 50 ng/ml PMA (Sigma, P8139) + 500 ng/ml ionomicin (Sigma I0634) in phenol red free RPMI 1640 medium + 10% fetal calf serum at 37°C with the addition of 10ng/ml Brefeldin A (Sigma, B7651) for a further 2 hr (Ferrick et al. 1995. *Nature* 373: 255-257). After washing, cells were fixed in 2% v/v formaldehyde in PBS (20min, 4°C), washed, and permeabilized with PBS + 0.5% saponin (Sigma S-2149). The following antibody conjugates were added in saponin buffer for 30 mins at 4°C, followed by extensive washing in saponin buffer followed by PBS + 0.1% azide + 1% BSA, + 5% HINRS: anti-IL-2 (S4B6-FITC; Pharmingen 18004A), anti-IL4 (11B11-FITC; Pharmingen 18194A), anti-IFN-γ (XMG1.2-FITC; Pharmingen 18114A). Cells were finally labelled with antibodies to surface CD4 and CD44, fixed in 1% formalin, and analysed on a FACSort as above. The conditions of stimulation, staining and analysis were such that normal CBA/Ca CD4⁺ spleen cells were essentially negative for all cytokine stains.

Treatment with CD4 monoclonal antibody

The non-depleting rat IgG2a anti-mouse CD4 (YTS 177.9 Qin et al. 1990) was made by growing the hybridoma in a hollow fibre bioreactor, and was purified under sterile and low endotoxin conditions by precipitation with 50% saturated ammonium sulfate. (These are standard techniques known to a person skilled in the art. An example of the technique is also available on <http://www.molbiol.ox.ac.uk/www/pathology/tig/mprod.html>). Grafted A1(M)xRAG^{-/-} mice were given 5 x 1mg intraperitoneally from the day of grafting over a two week period.

Results and Discussion

Analysis of A1(M) mice transgenic for TCR against H-Y+H2-E^k.

Thymus, spleens, and lymph nodes from A1(M) mice were analysed by 3 colour immunofluorescence to determine whether the expression of the transgenic TCR led to the predicted functional modification of the T cell repertoire. The thymi of female A1(M) mice were found to have a strong bias towards the generation of CD4⁺CD8⁻ rather than CD8⁺CD4⁻ T cells, as expected from an increased positive selection of the MHC-II restricted anti-H-Y TCR. This led to a CD4/CD8 ratio in the peripheral lymphoid organs in excess of 10:1, and expression of the V β 8.2 transgenic receptor on more than 90% of CD3⁺ cells. In contrast, male A1(M) mice had smaller thymi (a mean of 6×10^6 total thymocytes compared to 8×10^7 in females), with a mature CD4/CD8 ratio close to 1:1, and a similar expression of V β 8.2 to non-transgenic CBA/Ca mice, suggesting clonal deletion of anti-H-Y transgenic T cells and escape of endogenous TCR rearrangements. These A1(M) mice were then crossed onto a RAG-1^{-/-} background, to eliminate all B cells and T cells expressing other TCR molecules encoded by endogenous TCR rearrangements, so that any

ability of H-Y specific CD4⁺ T cells to reject male skin grafts could be unambiguously identified.

Positive selection in female and negative selection in male

5 A1(M)xRAG-1^{-/-} mice

Immunofluorescent staining of A1(M)xRAG^{-/-} mice confirmed that the anti-H-Y TCR was functional, as positive selection and the generation of CD3⁺CD4⁺CD8⁻ thymocytes was only observed in female thymi, while male thymi were much smaller, with very few CD4⁺CD8⁻ cells.

10 When we looked in more detail at these few CD4⁺CD8⁻ cells we found them present in similar numbers in both male A1xRAG-1^{-/-} and RAG-1^{-/-} controls, and that none of them expressed CD3 but were mostly CD11c⁺, suggesting they may be related to CD4⁺ immature dendritic cells (Winkel et al. 1994 *Immunol. Lett.* 40: 93-9) rather than T cells that had somehow escaped 15 deletion. Staining of lymph-nodes confirmed that only CD4⁺ and not CD8⁺ T cells were present in female A1(M)xRAG-1^{-/-}, and that clonal deletion in the male reduced the number of CD4⁺ cells down to that seen in RAG-1^{-/-} mice (and these were again CD3⁺CD11c⁺). The expression of the TCR in female A1(M)xRAG-1^{-/-}, as measured by CD3 or V β 8.2 staining, was lower 20 than in a normal CBA/Ca mouse (approx. 30% of the median fluorescence level), but similar to that of the A1(M) founders, which may be a property of the CD2 expression system (G. Stockinger, personal communication).

Rejection of male skin by female A1(M)xRAG^{-/-} mice

25 In initial experiments in two laboratories, a total of 8 female A1(M)xRAG-1^{-/-} mice were given single male skin grafts, four of which were rapidly rejected (within 16 days) and a further two were eventually rejected in a chronic fashion. Subsequently, a group of 5 female A1(M)xRAG-1^{-/-} mice were simultaneously grafted with male and female CBA/Ca skin in the 30 same graft bed. All of the male grafts were rapidly rejected (within 14

days), while the female grafts remained perfectly healthy (Figure 2). A second group of five was grafted in an identical fashion but were also treated with saturating amounts of a monoclonal antibody that blocks CD4 function *in vivo*. All these grafts were accepted, proving that the rejection 5 was both CD4 dependent and male specific. As illustrated in Table 1, non-depleting CD4 monoclonal antibodies produce an indefinite tolerant state (ability to accept fresh male skin grafts) even well after the antibodies are no longer in circulation.

10 **Mechanism of CD4 dependent graft rejection**

The A1(M)xRAG-1^{-/-} mice should have no CD8⁺ T cells or antibody producing B cells that might be able to act as effectors of graft rejection, and this was checked by staining spleen cells from two female A1(M)xRAG-1^{-/-} mice that had been allowed to reject two sequential male 15 grafts, and had been grafted with a third male skin 7 days previously, such that if there was any hypothetical expansion of, for example, a novel CD8⁺ population during graft rejection, this should become visible. However, it was confirmed that there was no CD3⁺CD8⁺ staining above background (Figure 3), and that CD25 expression was limited to the CD3⁺CD4⁺ subset: 20 Even if there had been some CD8⁺TCR⁺ cells that remained below the level of detection, these should have been unable to interact effectively with the H-2E^k presented male antigen, and would thus be extremely unlikely to contribute to graft rejection. Similarly, there were no surface Ig⁺ B cells that might have been able to contribute an antibody response. 25 There was clear evidence that the male graft was indeed being recognized by the transgenic anti-male TCR on CD4⁺ T cells, as the majority (approx. 70%) of these could be shown to be recently memory or recently activated cells (expressing CD44) as well as producing both IFN- γ , and to a lesser extent, IL-4 (Figure 3). Interestingly, all T cells (both CD44⁺ and CD44⁻) 30 appeared to be expressing IL-2 by this method of intracytoplasmic staining.

It is therefore clear that the transgenic TCR⁺CD4⁺ T cells in A1(M)xRAG-1^{-/-} females are sufficient to reject male skin. The mechanism by which the animals are made tolerant by non-depleting CD4 antibodies is neither deletion nor a conversion of Th1 to Th2 cells. The tolerisation may 5 be due to a direct, CD4⁺ T cell mediated cytotoxicity or it may be due to an indirect, perhaps cytokine mediated, help for macrophages or another antigen non-specific effector cell. Recent data suggesting that neither Fas/FasL nor perforin (Selvaggi et al. 1996 *Transplantation*. 62(12): 1912-5) are required for CD4 mediated rejection of MHC-I disparate skin would 10 tend to favour the latter hypothesis. The MHC-I restricted CD8⁺ arm of the immune response in normal mice would therefore seem to be mostly dependent on (Antoniou et al. 1996 *Eur. J. Immunol.* 26(5): 1094-1020), and an amplification of, the MHC-II restricted CD4⁺ response that we have shown here can itself provide all the necessary T cell functionality for graft 15 rejection.

Example 2 – Testing of reagents for Immunosuppressive or
Tolerogenic effects in A1(M) x RAG^{-/-} mice

A variety of different agents was tested. The testing methods 20 used were as follows and results are given in Table 1.

- a) Female A1xRAG-1^{-/-} mice were given 10⁷ male A1xRAG-1^{-/-} bone marrow or spleen cells intravenously. They were then grafted with male tail skin after a further 6 weeks to test for tolerance, which was accepted indefinitely. Immunofluorescent analysis of peripheral 25 blood and spleen cells indicated substantial deletion of the CD4+ T cells in the tolerant mice, but not in non tolerant controls that had been given female marrow or spleen cells.
- b) Female A1xRAG-1^{-/-} mice were given 5 x 1mg or a single injection of 1mg of non-depleting rat IgG2a anti mouse CD4 (YTS 177.9 Qin 30 et al 1990 *Eur. J Immunol* 20: 2737-2745) at the time of grafting

male CBA/Ca tail skin. These grafts and subsequent male skin grafted at 42 and 84 days were accepted indefinitely. Analysis of blood, lymph-nodes and spleen cells from these mice demonstrated that CD4+ T cells had not been clonally deleted, and similar proportions of Th1 and Th2 cells could be identified by immunofluorescent staining for cytokines in both the tolerant mice and control animals that had not been treated with CD4 antibody but had received equivalent male skin grafts.

- 10 c) Female A1xRAG-1^{-/-} mice were given 5 x 1mg of monoclonal antibody to CD25 (PC61: Osawa et al., Immunol. lett. 1989 20: 205-12) from the time of grafting with male CBA/Ca tail skin.

d) Female A1xRAG-1^{-/-} mice were given 5 x 1mg of monoclonal antibody to CD40 ligand (MR1: Larsen et al. Transplantation 1996 61: 4-9) from the time of grafting with male CBA/Ca tail skin.

15 e) Female A1xRAG-1^{-/-} mice were given 5 x 1mg of monoclonal antibody to CD28 (37.51: Sperling et al., J. Immunol. 1993 151: 6043-50) from the time of grafting with male CBA/Ca tail skin.

Table 1

Examples of testing reagents for immunosuppressive or tolerogenic effects in the Toleramouse

	Agent tested	Source	No. Tested	Effect	Mechanism	Comments
10	Male antigen iv.	Bone marrow	6	Full tolerance to male skin	Predominantly clonal deletion	Model for blood transfusion effect
	Male antigen iv.	Spleen cells	6	Full tolerance to male skin	Predominantly clonal deletion	Model for blood transfusion effect
15	Non-depleting CD4 monoclonal antibody	YTS 177.9	6	Full tolerance to male skin	Not deletion and not Th1 -> Th2	Accepted 2nd and 3rd male skin grafts
	Anti-CD25 monoclonal antibody	PC61	6	Immuno-suppression	Not done	Rejection delayed
20	Anti-CD40 ligand monoclonal antibody	MR1	6	None	Not applicable	Dose regimen may not have been optimal
	Anti-CD28 monoclonal antibody	37.51	3	None	Not applicable	Antibody preparation may not have been active
30						

Example 3 - Construction of A1(M)xRAG-1^{-/-} mouse from sequence information only

35 Making TCR transgenic mice is a routine procedure where the appropriate TCR alpha and beta chain encoding DNA is micro-injected into fertilized eggs from the strain of mice in which it is desired to express the TCR (for example the standard Olac CBA/Ca strain). The injected eggs are then transferred into foster mothers, and the offspring typed by 40 standard methods (usually by PCR typing of tail DNA) to select those which have stably incorporated the DNA into their genome. These are further checked to ensure the DNA is expressed as a functional TCR, using standard immunological techniques (staining with antibodies, T cell proliferation to antigen etc.). These A1(M) transgenic mice are then 45 crossed with RAG-1^{-/-} deficient mice (Alt et al. 1992 and Mombaerts et al. 1992), and the offspring are backcrossed onto the A1(M) parents for a

number of generations, selecting those that carry both the TCR and RAG-¹⁺ genes.

The A1 TCR DNA can be made in a number of ways; including the following:

5

- 1) By cloning the TCR alpha and beta chain mRNAs as cDNA (standard method) from an original A1 CD4⁺ T cell clone such as the A1 CD4⁺ anti-H-Y T cell clone.
- 10 2) By cloning the TCR alpha and beta chain mRNAs as cDNA (standard method) from the A1(M) or A1(M)xRAG-1⁺ female mice or CD4⁺ anti-H-Y T cell clones derived from them.
- 15 3) By using the DNA sequence information to synthesize the TCR alpha and beta chain genes. This can simply be performed by making a series of overlapping oligonucleotides of convenient length (eg. 30-50 bases each) such that the entire sequence on both strands is covered. Simple annealing and ligation then generates the full length, double stranded DNA for each gene. This method requires only suitable TCR sequence information (for example the sequence in figure 1) to generate the A1(M) mice, using techniques well known in the art of making transgenic mice.
- 20 4) By using the DNA sequence information to mutate (standard methods of site-directed mutagenesis) other (related) TCR alpha and beta chain genes to a known desired sequence (either the entire sequence or just the complementarity determining regions (CDRs) that define the antigen binding site) such as the TCR gene sequences shown in figure 1.

Once the TCR alpha and beta chain genes have either been cloned or synthesized they are ligated into an appropriate expression vector or cassette that allows them to be expressed in T cells. The CD2

minigene cassette system described in Zhumabekov et al. 1995 *J. Immunol. Methods* 185(1):133-40 is a suitable expression system.

Legends to Figures

5

Figure 1 - A1 TCR α and β chains and nucleic acid sequences encoding them.

10 **Figure 2 - Female A1(M)xRAG-1 $^{-/-}$ mice show CD4 dependent, specific rejection of male skin**

Female A1(M)xRAG-1 $^{-/-}$ mice were grafted with male and female CBA/Ca skin in the same graft bed. Survival plots are shown for the male skin that rejected with a median survival time (MST) of 14 days (●; n=5) compared to the accompanying female grafts that survived beyond day 30 (○ ; n=5). The P value for statistical significance was <0.003 (LogRank method). Also shown is the survival of male skin on similarly grafted A1(M)xRAG-1 $^{-/-}$ female mice treated with 5 x 1mg non-depleting CD4 antibody (■ ; n=5; MST >30 days).

20 **Figure 3 - Phenotypic and functional immunofluorescent analysis of rejecting A1(M)xRAG $^{-/-}$ mice**

Two female A1(M)xRAG-1 $^{-/-}$ mice, that had rejected two sequential male skin grafts were given a third graft, and their spleen cells stained for a number of surface and intracytoplasmic antigens.

25 Representative examples of four colour immunofluorescent analysis from one of the mice are shown. All samples were live gated on forward and side scatters, and the dot plot in the upper left panel shows CD4-PE versus CD8 α -QR staining of the CD3-FITC positive lymphocytes. The upper right panel shows that there were no B cells expressing surface Ig in the A1(M)xRAG-1 $^{-/-}$ (filled histogram) compared to an A1(M) control (broken

30

line histogram). The centre left panel shows the staining for CD44-QR of A1(M)xRAG-1⁻ lymphocytes, that was used as the basis for gating the remaining anti-cytokine stains (rat IgG1 anti-IFN- γ , centre right; rat IgG1 anti-IL-2, lower left; rat IgG1 anti-IL-4, lower right), where the CD44⁺ cells are shown as filled histograms, the CD44⁻ cells as open histograms, and the negative control histogram (based on the background staining of isotype matched, rat IgG1 anti-IL4-FITC in normal mice) is shown with a broken line.

CLAIMS

1. A genetically modified non-human mammal having a population of CD4 positive T cells specific for one or a limited number of selected antigens, including at least one transplantation antigen, the 5 mammal being capable of an immune response against the transplantation antigen and of being tolerised to the transplantation antigen.
2. The genetically modified mammal according to claim 1, which is capable of rejecting a tissue transplant containing the transplantation antigen and the other selected antigens.
- 10 3. The genetically modified mammal according to claim 1 or claim 2, having stably integrated into its genome T cell receptor genes which encode a T cell receptor specific for the transplantation antigen.
4. The genetically modified mammal according to any one of claims 1 to 3, which lacks a normal population of CD8 positive T cells or B 15 cells or both.
5. The genetically modified mammal according to claim 4, having no functional CD8 positive T cells or B cells.
6. The genetically modified mammal according to any one of claims 1 to 5, wherein there is a deficiency in lymphocyte receptor 20 recombination such that no T cell or B cell receptors are expressed other than the T cell receptor for the transplantation antigen and if present, T cell receptors for the limited number of other selected antigens.
7. The genetically modified mammal according to any one of claims 1 to 6, wherein a gene involved in lymphocyte receptor 25 recombination is inactivated or deleted.
8. The genetically modified mammal according to claim 7, wherein one or both copies of the RAG-1 and/or RAG-2 gene are inactivated or deleted.

9. The genetically modified mammal according to any one of claims 1 to 8, wherein the transplantation antigen is a male or female transplantation antigen.
10. The genetically modified mammal according to claim 9,
5 wherein the transplantation antigen is the male transplantation antigen H-Y.
11. The genetically modified mammal according to claim 10,
wherein the T cell receptor specific for H-Y is encoded by the nucleic acid sequences shown in figure 1.
- 10 12. The genetically modified mammal according to any one of claims 1 to 11, for use in studying tolerance.
13. The genetically modified mammal according to any one of claims 1 to 12, for use in drug screening.
14. A method of screening for biologically active compounds
15 which method comprises administering the compounds to a genetically modified non-human mammal according to any one of claims 1 to 13 and observing the effect of the compounds on the ability of the mammal to reject or maintain a transplant containing the transplantation antigen.
15. The method according to claim 14, comprising the further
20 step of employing a biologically active compound identified by the method, as a tolerance enhancing or inducing or suppressing agent.
16. A method which comprises inducing or enhancing or suppressing tolerance by means of a biologically active compound identified by the screening method according to claim 14.
- 25 17. A method of investigating immunological changes in tolerance or rejection, which method comprises applying a suitable tissue transplant to a mammal according to any one of claims 1 to 13 under conditions to promote tolerance or rejection, and monitoring changes in one or more immunological indicators such as cytokine levels or T cell activation markers.

18. A method of studying transplantation tolerance or rejection, which method comprises applying a suitable tissue transplant to an animal according to any one of claims 1 to 13 under conditions to promote tolerance to or rejection of the transplant, and assessing biological changes in the animal.

19. A method of studying immunological tolerance in pregnancy, which method comprises providing a pregnant female animal according to any one of claims 1 to 13, said female animal carrying at least one foetus displaying the transplantation antigen, and monitoring changes in one or more immunological indicators such as cytokine levels or T cell activation markers.

Figure 1A1 TCR alpha chain [SEQ ID NO: 5 and 6]

5 MKRLLCSLLGLLCTQVCWVKGQQVQQSPASLVHQEGENAEIQCNFSTSLNSMQWFYQRPEGSLVSL
 FYNPSGTQSGRLTSTVIKERRSSLHISSSQITDSGYLCADWTGNTRKLIFGLGTTLQVQPDIQ
 NPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKTVLDMKAMDSKSNGAIAWSNQT
 SFTCQDIFKETNATYPSSDVPCDATLTEKSFETDMNLNFQNLSVMGLRILLKVAGFNLLMTLRLW
 SS

10 acaagcacca tgaagaggct gctgtgctct ctgctgggc ttctgtgcac ccaggttgc 60
 tgggtgaaag gacagcaagt gcagcagac cccgcgtcct tggttctgca ggagggggag 120
 aatgcagacg tgcagtgtaa cttttccaca tcattgaaca gtatgcagtg gttttaccaa 180
 cgtcctgagg gaagtctcgta cagcctgttc tacaatcctt ctgggacaaa gcagagtggg 240

15 agactgacat ccacaacagt catcaaagaa cgtcgcagct cttgcacat ttccctcc 300
 cagatcacag actcaggcac ttatctctgt gccgattgga caggcaatac tagaaaactc 360
 atcttgggc tggggacaac ttacaagtg caaccagaca tccagaaccc agaacctgct 420
 gtgtaccagt taaaagatcc tcggtctcgta gacagcaccc tctgcctgtt caccgacttt 480
 gactccaaa tcaatgtgcc gaaaaccatg gaatctggaa cgttcatcac tgacaaaact 540

20 gtgctggaca taaaagctat ggattccaag agcaatgggg ccattgcctg gagcaaccag 600
 acaagcttca cctgccaaga tatcttcaaa gagaccaacg ccacacctccc cagttcagac 660
 gttccctgtg atgccacgtt gaccgagaaa agcttgaaa cagatatgaa cctaaacttt 720
 caaaacctgt cagttatggg actccgcac tcctgcgtga aagtagcggg atttaacctg 780
 ctcatgacgc tgaggctgtg gtccagttga ggtct 815

25

A1 TCR beta chain [SEQ ID NO: 7 and 8]

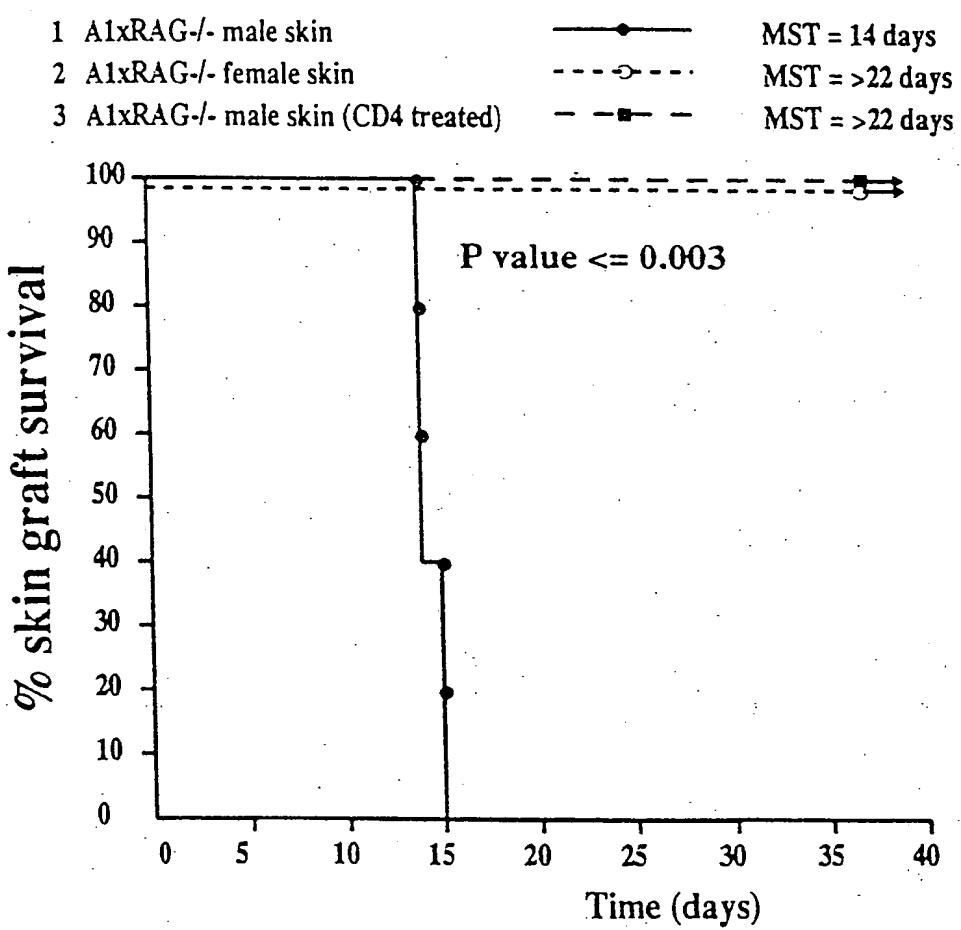
MSNTAFPDPAWNTLLSVALFLLGKHMEEAVTQSPRNKAVTGGKVTLSNCQTNNHNNMYWYRQ
 DTGHGLRLIHYSYGAGSTEKGDIQDGYKASRPSQENFSLILELATPSQTSVYFCASGDGLGSETL
 YFGSGTRLTVLEDLRNVTPPKVSLFEPSKAEIANKQKATLVCLARGFFPDHVVELSWWVNGKEVHSG

30 VSTDPOAYKESNYSYCLSSRLRVSATFWHNPRNHRCQVFHGLSEEDKWPEGSPKPVHRTSVQRP
 GAEQCGITSSYHQGVLSATILYEILLGKATLYAVLVSGLVLMAVKKNS

	agaggaagca tgtctaacac tgccttcctt gacccgcctt ggaacaccac cctgctatct	60
	tgggttgctc tctttctcctt gggaaacaaaa cacatggagg ctgcagtac cc当地agccaa	120
	agaaaacaagg tggcagtaac aggaggaaag gtgacattga gctgtaatca gactataac	180
	cacaacaaca tgtactggta tcggcaggac acggggcatg ggctgaggct gatccattat	240
5	tcatatggta ctggcagcac tgagaaagga gatatccctg atggatacaa ggcctccaga	300
	ccaagccaa agaacttctc cctcattctg gagttggcta ccccctctca gacatcagt	360
	tacttctgtg ccagcggtga ttccggactg gggtcagaaa cgctgtattt tggctcagga	420
	accagactga ctgttctcga ggatctgaga aatgtgactc cacccaaaggctt ctccctt	480
	gagccatcaa aagcagagat tgcaaacaaca caaaaggcta ccctcgtgtg cttggccagg	540
10	ggcttcttcc ctgaccacgt ggagctgagc tgggtgggtga atggcaagga ggtccacagt	600
	ggggtcagca cggaccctca ggcctacaag gagagcaatt atagctactg cctgagcagc	660
	cgcctgaggg tctctgctac cttctggcac aatctcgaa accacttccg ctgccaagt	720
	cagttccatg ggcttcaga ggaggacaag tggccagagg gctcacccaa acctgtacac	780
	agaacatcag tgcaaggagcc tggggccgag cagtgtggaa tcacttcatc ctatcatcag	840
15	ggggttctgt ctgcaaccat cctctatgag atcctactgg ggaaggccac cctatatgct	900
	gtgctggta gtggcctagt gctgatggcc atggtaaga aaaaaaattc ctga	954

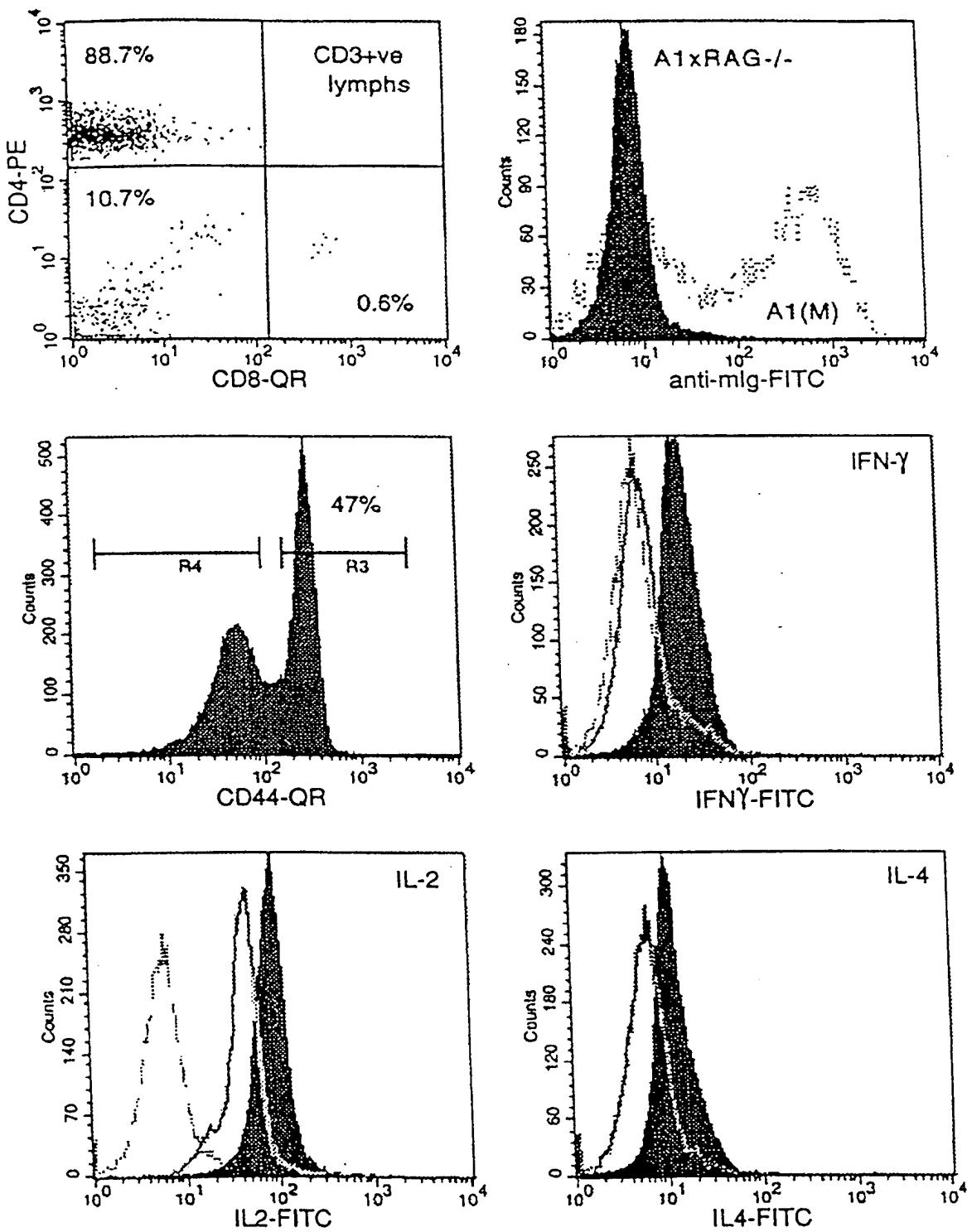
3 / 4

Figure 2



4 / 4

Figure 3



1

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: ISIS INNOVATION LIMITED
- (B) STREET: 2 SOUTH PARKS ROAD
- (C) CITY: OXFORD
- (D) STATE: OXON
- (E) COUNTRY: UNITED KINGDOM
- (F) POSTAL CODE (ZIP): OX1 3UB

(ii) TITLE OF INVENTION: TOLERANCE

(iii) NUMBER OF SEQUENCES: 8

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: GB 9720888.8

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GCGAATCACA AGCACCATGA AGAGGCTG

28

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GCGAATTCCA GACCTCAACT GGACCACAG

29

2

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GCGAATTCAAG AGGAAGCATG TCTAACACT

29

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GCGAATTCAAG GATGCATAAA AGTTTGTCTC AGG

33

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 815 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Mus musculus*

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 10..810
- (D) OTHER INFORMATION: /codon_start= 1
/product= "A1 TCR alpha chain"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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Met Lys Arg Leu Leu Cys Ser Leu Leu Gly Leu Leu Cys

1

5

10

48

.3

ACC CAG GTT TGC TGG GTG AAA GGA CAG CAA GTG CAG CAG AGC CCC GCG		96
Thr Gln Val Cys Trp Val Lys Gly Gin Gln Val Gln Gln Ser Pro Ala		
15	20	25
TCC TTG GTT CTG CAG GAG GGG GAG AAT GCA GAG CTG CAG TGT AAC TTT		144
Ser Leu Val Leu Gln Glu Gly Glu Asn Ala Glu Leu Gln Cys Asn Phe		
30	35	40
45		
TCC ACA TCT TTG AAC AGT ATG CAG TGG TTT TAC CAA CGT CCT GAG GGA		192
Ser Thr Ser Leu Asn Ser Met Gln Trp Phe Tyr Gln Arg Pro Glu Gly		
50	55	60
55		
AGT CTC GTC AGC CTG TTC TAC AAT CCT TCT GGG ACA AAG CAG AGT GGG		240
Ser Leu Val Ser Leu Phe Tyr Asn Pro Ser Gly Thr Lys Gln Ser Gly		
65	70	75
75		
AGA CTG ACA TCC ACA ACA GTC ATC AAA GAA CGT CGC AGC TCT TTG CAC		288
Arg Leu Thr Ser Thr Thr Val Ile Lys Glu Arg Arg Ser Ser Leu His		
80	85	90
90		
ATT TCC TCC TCC CAG ATC ACA GAC TCA GGC ACT TAT CTC TGT GCC GAT		336
Ile Ser Ser Ser Gln Ile Thr Asp Ser Gly Thr Tyr Leu Cys Ala Asp		
95	100	105
105		
TGG ACA GGC AAT ACT AGA AAA CTC ATC TTT GGG CTG GGG ACA ACT TTA		384
Trp Thr Gly Asn Thr Arg Lys Leu Ile Phe Gly Leu Gly Thr Thr Leu		
110	115	120
120		125
CAA GTG CAA CCA GAC ATC CAG AAC CCA GAA CCT GCT GTG TAC CAG TTA		432
Gln Val Gln Pro Asp Ile Gln Asn Pro Glu Pro Ala Val Tyr Gln Leu		
130	135	140
140		
AAA GAT CCT CGG TCT CAG GAC AGC ACC CTC TGC CTG TTC ACC GAC TTT		480
Lys Asp Pro Arg Ser Gln Asp Ser Thr Leu Cys Leu Phe Thr Asp Phe		
145	150	155
155		
GAC TCC CAA ATC AAT GTG CCG AAA ACC ATG GAA TCT GGA ACG TTC ATC		528
Asp Ser Gln Ile Asn Val Pro Lys Thr Met Glu Ser Gly Thr Phe Ile		
160	165	170
170		
ACT GAC AAA ACT GTG CTG GAC ATG AAA GCT ATG GAT TCC AAG AGC AAT		576
Thr Asp Lys Thr Val Leu Asp Met Lys Ala Met Asp Ser Lys Ser Asn		
175	180	185
185		
GGG GCC ATT GCC TGG AGC AAC CAG ACA AGC TTC ACC TGC CAA GAT ATC		624
Gly Ala Ile Ala Trp Ser Asn Gln Thr Ser Phe Thr Cys Gln Asp Ile		
190	195	200
200		205
TTC AAA GAG ACC AAC GCC ACC TAC CCC AGT TCA GAC GTT CCC TGT GAT		672
Phe Lys Glu Thr Asn Ala Thr Tyr Pro Ser Ser Asp Val Pro Cys Asp		
210	215	220
220		
GCC ACG TTG ACC GAG AAA AGC TTT GAA ACA GAT ATG AAC CTA AAC TTT		720
Ala Thr Leu Thr Glu Lys Ser Phe Glu Thr Asp Met Asn Leu Asn Phe		
225	230	235
235		

4

CAA AAC CTG TCA GTT ATG GGA CTC CGC ATC CTC CTG CTG AAA GTA GCG	768	
Gln Asn Leu Ser Val Met Gly Leu Arg Ile Leu Leu Lys Val Ala		
240	245	250
GGA TTT AAC CTG CTC ATG ACG CTG AGG CTG TGG TCC AGT TGA	810	
Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser *		
255	260	265
GGTCT	815	

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 267 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met Lys Arg Leu Leu Cys Ser Leu Leu Gly Leu Leu Cys Thr Gln Val			
1	5	10	15

Cys Trp Val Lys Gly Gln Gln Val Gln Gln Ser Pro Ala Ser Leu Val		
20	25	30

Leu Gln Glu Gly Glu Asn Ala Glu Leu Gln Cys Asn Phe Ser Thr Ser		
35	40	45

Leu Asn Ser Met Gln Trp Phe Tyr Gln Arg Pro Glu Gly Ser Leu Val		
50	55	60

Ser Leu Phe Tyr Asn Pro Ser Gly Thr Lys Gln Ser Gly Arg Leu Thr			
65	70	75	80

Ser Thr Thr Val Ile Lys Glu Arg Arg Ser Ser Leu His Ile Ser Ser		
85	90	95

Ser Gln Ile Thr Asp Ser Gly Thr Tyr Leu Cys Ala Asp Trp Thr Gly		
100	105	110

Asn Thr Arg Lys Leu Ile Phe Gly Leu Gly Thr Thr Leu Gln Val Gln		
115	120	125

Pro Asp Ile Gln Asn Pro Glu Pro Ala Val Tyr Gln Leu Lys Asp Pro		
130	135	140

Arg Ser Gln Asp Ser Thr Leu Cys Leu Phe Thr Asp Phe Asp Ser Gln			
145	150	155	160

Ile Asn Val Pro Lys Thr Met Glu Ser Gly Thr Phe Ile Thr Asp Lys		
165	170	175

Thr Val Leu Asp Met Lys Ala Met Asp Ser Lys Ser Asn Gly Ala Ile		
180	185	190

Ala Trp Ser Asn Gln Thr Ser Phe Thr Cys Gln Asp Ile Phe Lys Glu
 195 200 205

Thr Asn Ala Thr Tyr Pro Ser Ser Asp Val Pro Cys Asp Ala Thr Leu
 210 215 220

Thr Glu Lys Ser Phe Glu Thr Asp Met Asn Leu Asn Phe Gln Asn Leu
 225 230 235 240

Ser Val Met Gly Leu Arg Ile Leu Leu Lys Val Ala Gly Phe Asn
 245 250 255

Leu Leu Met Thr Leu Arg Leu Trp Ser Ser *
 260 265

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 954 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Mus musculus*

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 10..954
- (D) OTHER INFORMATION: /codon_start= 1
/product= "A1 TCR beta chain"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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270 275 280	
ACC CTG CTA TCT TGG GTT GCT CTC TTT CTC CTG GGA ACA AAA CAC ATG	96
Thr Leu Leu Ser Trp Val Ala Leu Phe Leu Leu Gly Thr Lys His Met	
285 290 295	
GAG GCT GCA GTC ACC CAA AGC CCA AGA AAC AAG GTG GCA GTA ACA GGA	144
Glu Ala Ala Val Thr Gln Ser Pro Arg Asn Lys Val Ala Val Thr Gly	
300 305 310	
GGA AAG GTG ACA TTG AGC TGT AAT CAG ACT AAT AAC CAC AAC AAC ATG	192
Gly Lys Val Thr Leu Ser Cys Asn Gln Thr Asn Asn His Asn Asn Met	
315 320 325	
TAC TGG TAT CGG CAG GAC ACG GGG CAT GGG CTG AGG CTG ATC CAT TAT	240
Tyr Trp Tyr Arg Gln Asp Thr Gly His Gly Leu Arg Leu Ile His Tyr	
330 335 340	

6

TCA TAT GGT GCT GGC AGC ACT GAG AAA GGA GAT ATC CCT GAT GGA TAC Ser Tyr Gly Ala Gly Ser Thr Glu Lys Gly Asp Ile Pro Asp Gly Tyr 345	350	355	360	288
AAG GCC TCC AGA CCA AGC CAA GAG AAC TTC TCC CTC ATT CTG GAG TTG Lys Ala Ser Arg Pro Ser Gln Glu Asn Phe Ser Leu Ile Leu Glu Leu 365	370	375		336
GCT ACC CCC TCT CAG ACA TCA GTG TAC TTC TGT GCC AGC GGT GAT TCG Ala Thr Pro Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Gly Asp Ser 380	385	390		384
GGA CTG GGG TCA GAA ACG CTG TAT TTT GGC TCA GGA ACC AGA CTG ACT Gly Leu Gly Ser Glu Thr Leu Tyr Phe Gly Ser Gly Thr Arg Leu Thr 395	400	405		432
GTT CTC GAG GAT CTG AGA AAT GTG ACT CCA CCC AAG GTC TCC TTG TTT Val Leu Glu Asp Leu Arg Asn Val Thr Pro Pro Lys Val Ser Leu Phe 410	415	420		480
GAG CCA TCA AAA GCA GAG ATT GCA AAC AAA CAA AAG GCT ACC CTC GTG Glu Pro Ser Lys Ala Glu Ile Ala Asn Lys Gln Lys Ala Thr Leu Val 425	430	435	440	528
TGC TTG GCC AGG GGC TTC TTC CCT GAC CAC GTG GAG CTG AGC TGG TGG Cys Leu Ala Arg Gly Phe Phe Pro Asp His Val Glu Leu Ser Trp Trp 445	450	455		576
GTG AAT GGC AAG GAG GTC CAC AGT GGG GTC AGC ACG GAC CCT CAG GCC Val Asn Gly Lys Glu Val His Ser Gly Val Ser Thr Asp Pro Gln Ala 460	465	470		624
TAC AAG GAG AGC AAT TAT AGC TAC TGC CTG AGC AGC CGC CTG AGG GTC Tyr Lys Glu Ser Asn Tyr Ser Tyr Cys Leu Ser Ser Arg Leu Arg Val 475	480	485		672
TCT GCT ACC TTC TGG CAC AAT CCT CGA AAC CAC TTC CGC TGC CAA GTG Ser Ala Thr Phe Trp His Asn Pro Arg Asn His Phe Arg Cys Gln Val 490	495	500		720
CAG TTC CAT GGG CTT TCA GAG GAG GAC AAG TGG CCA GAG GGC TCA CCC Gln Phe His Gly Leu Ser Glu Glu Asp Lys Trp Pro Glu Gly Ser Pro 505	510	515	520	768
AAA CCT GTA CAC AGA ACA TCA GTG CAG AGG CCT GGG GCC GAG CAG TGT Lys Pro Val His Arg Thr Ser Val Gln Arg Pro Gly Ala Glu Gln Cys 525	530	535		816
GGA ATC ACT TCA TCC TAT CAT CAG GGG GTT CTG TCT GCA ACC ATC CTC Gly Ile Thr Ser Ser Tyr His Gln Gly Val Leu Ser Ala Thr Ile Leu 540	545	550		864
TAT GAG ATC CTA CTG GGG AAG GCC ACC CTA TAT GCT GTG CTG GTC AGT Tyr Glu Ile Leu Leu Gly Lys Ala Thr Leu Tyr Ala Val Leu Val Ser 555	560	565		912

GGC CTA GTG CTG ATG GCC ATG GTC AAG AAA AAA AAT TCC TGA 954
 Gly Leu Val Leu Met Ala Met Val Lys Lys Lys Asn Ser *
 570 575 580

(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:

 - (A) LENGTH: 315 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ_ID_NO: 8:

Met	Ser	Asn	Thr	Ala	Phe	Pro	Asp	Pro	Ala	Trp	Asn	Thr	Thr	Leu	Leu
1	.				5					10				15	

Ser Trp Val Ala Leu Phe Leu Leu Gly Thr Lys His Met Glu Ala Ala
20 25 30

Thr Leu Ser Cys Asn Gln Thr Asn Asn His Asn Asn Met Tyr Trp Tyr
50 55 60

Arg Gln Asp Thr Gly His Gly Leu Arg Leu Ile His Tyr Ser Tyr Gly
65 70 75 80

Ala Gly Ser Thr Glu Lys Gly Asp Ile Pro Asp Gly Tyr Lys Ala Ser
85 90 95

Arg Pro Ser Gln Glu Asn Phe Ser Leu Ile Leu Glu Leu Ala Thr Pro
100 105 110

Ser Gln Thr Ser Val Tyr Phe Cys Ala Ser Gly Asp Ser Gly Leu Gly
115 120 125

Ser Glu Thr Leu Tyr Phe Gly Ser Gly Thr Arg Leu Thr Val Leu Glu
 130 135 140 . .

Asp Leu Arg Asn Val Thr Pro Pro Lys Val Ser Leu Phe Glu Pro Ser
145 150 155 160

Lys Ala Glu Ile Ala Asn Lys Gln Lys Ala Thr Leu Val Cys Leu Ala
165 170 175

Arg Gly Phe Phe Pro Asp His Val Glu Leu Ser Trp Trp Val Asn Gly
180 185 190

Lys Glu Val His Ser Gly Val Ser Thr Asp Pro Gln Ala Tyr Lys Glu
195 200 205

Ser Asn Tyr Ser Tyr Cys Leu Ser Ser Arg Leu Arg Val Ser Ala Thr
210 215 220

Phe Trp His Asn Pro Arg Asn His Phe Arg Cys Gln Val Gln Phe His
225 230 235 240

Gly Leu Ser Glu Glu Asp Lys Trp Pro Glu Gly Ser Pro Lys Pro Val
245 250 255

His Arg Thr Ser Val Gln Arg Pro Gly Ala Glu Gln Cys Gly Ile Thr
260 265 270

Ser Ser Tyr His Gln Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile
275 280 285

Leu Leu Gly Lys Ala Thr Leu Tyr Ala Val Leu Val Ser Gly Leu Val
290 295 300

Leu Met Ala Met Val Lys Lys Lys Asn Ser *
305 310 315

INTERNATIONAL SEARCH REPORT

Inte...onal Application No

PCT/GB 98/02965

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/00 A01K67/027 C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A01K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCULLY, R. ET AL.: "A role for Th2 cytokines in the suppression of CD8+ T cell-mediated graft rejection" EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 27, no. 7, July 1997, pages 1663-1670, XP002091475 MONTREUIL FR see page 1663, column 2, paragraph 2 ---	1,3
X	HÄMMERLING, G.J. ET AL.: "Peripheral tolerance as a multi-step mechanism" IMMUNOLOGICAL REVIEWS, vol. 133, 1993, pages 93-104, XP002091476 cited in the application see the whole document ---	1,3,12, 17,18 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

28 January 1999

18/02/1999

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Chambonnet, F

INTERNATIONAL SEARCH REPORT

Inte
lational Application No
PCT/GB 98/02965

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOUSKOFF V ET AL: "Cassette vectors directing expression of T cell receptor genes in transgenic mice" JOURNAL OF IMMUNOLOGICAL METHODS, vol. 180, no. 2, 27 March 1995, page 273-280 XP004021050 see page 276, column 2, line 1 ---	1,9,10
A	WO 90 06359 A (GENPHARM INT) 14 June 1990 see the whole document ---	1-7
A	WO 95 32285 A (INST NAT SANTE RECH MED ;UNIV PASTEUR (FR); CENTRE NAT RECH SCIENT) 30 November 1995 see the whole document ---	1
A	WO 97 08303 A (UNIV TECHNOLOGIES INT) 6 March 1997 see the whole document ---	1
A	MARSHALL, S. E. ET AL.: "Tolerance and suppression in a primed immune system" TRANSPLANTATION, vol. 62, no. 11, 15 December 1996, pages 1614-1621, XP002091477 see the whole document ---	1
A	COBBOLD, S.P. ET AL.: "Mechanisms of peripheral tolerance and suppression induced by monoclonal antibodies to CD4 and CD8" IMMUNOLOGICAL REVIEWS, vol. 0, no. 149, 1996, pages 5-33, XP002091478 cited in the application see the whole document ---	1
P, X	ZELENIKA, D. ET AL.: "Rejection of H-Y disparate skin grafts by monospecific CD4+ T helper 1 (Th1) and T helper 2 (Th2) cells : no requirement for CD8+T cells or B cells" JOURNAL OF IMMUNOLOGY, vol. 161, no. 4, 15 August 1998, pages 1868-1874, XP002091479 see the whole document ---	1
A, P	WO 97 35991 A (UNIV JOHNS HOPKINS ;SCHNECK JONATHAN P (US); HERRIN SEAN O (US)) 2 October 1997 see the whole document -----	1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 98/ 02965

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of Invention is lacking(Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

As far as claim 16 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

Although claims 17 and 18 are directed to surgical methods applied to the human/animal body, the search has been carried out and based on the alleged effects of the composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/02965

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9006359	A 14-06-1990	US 5175384 A		29-12-1992
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